Video Article

Lab-Scale Model to Evaluate Odor and Gas Concentrations Emitted by Deep Bedded Pack Manure

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Abstract

A lab-scaled simulated bedded pack model was developed to study air quality and nutrient composition of deep-bedded packs used in cattle mono-slope facilities. This protocol has been used to effectively evaluate many different bedding materials, environmental variables (temperature, humidity), and potential mitigation treatments that can improve air quality in commercial deep-bedded mono-slope facilities. The model is dynamic and allows researchers to easily collect many chemical and physical measurements from the bedded pack. Weekly measurements, collected over the course of six to seven weeks, allows sufficient time to see changes in air quality measurements over time as the bedded pack matures. The data collected from the simulated bedded packs is within the range of concentrations previously measured in commercial deep-bedded mono-slope facilities. Past studies have demonstrated that 8 - 10 experimental units per treatment are sufficient to detect statistical differences among the simulated bedded packs. The bedded packs are easy to maintain, requiring less than 10 minutes of labor per bedded packs per week to add urine, feces, and bedding. Sample collection using the gas sampling system requires 20 - 30 minutes per bedded pack, depending on the measurements that are being collected. The use of lab-scaled bedded packs allows the researcher to control variables such as temperature, humidity, and bedding source that are difficult or impossible to control in a research or commercial facility. While not a perfect simulation of "real-world" conditions, the simulated bedded packs serve as a good model for researchers to use to examine treatment differences among bedded packs. Several lab-scale studies can be conducted to eliminate possible treatments before trying them in a research or commercial-sized facility.
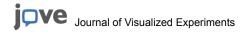
Video Link

The video component of this article can be found at https://www.jove.com/video/57332/

Introduction

Beef cattle confinement facilities are a popular housing option in the Midwest and Upper Great Plains. Confinement facilities are more common in this region than the Southern Plains because the region receives more annual precipitation, which creates more feedlot runoff that must be contained. Many producers chose to build mono-slope barns for beef cattle. The primary reasons cited by producers for selecting a mono-slope facility was the ability to schedule labor and manure removal, and improved performance compared to open lot feedlots[1]. A majority of cattle producers (72.2%) using mono-slope barns maintain a bedded pack for one turn of cattle or longer, using a deep-bedding management system for bedding and waste[1]. The most common bedding material used is corn stover, although producers report using soybean stubble, wheat straw, corn cobs, and sawdust[1]. Because of the regional demand for corn stover bedding, many producers were interested in alternative bedding materials that could be used in mono-slope facilities. In addition to economics and animal comfort, producers questioned how the bedding material would impact the environment of the facility, including the production of odorous gases, nutrient composition of the resulting manure/bedding, and presence of pathogens.

Few studies have been conducted to measure air quality resulting from different bedding materials used in livestock housing, with most focusing only on ammonia. Most of the previous evaluations of air quality include on-farm data collection with one or two experimental units per treatments being analyzed at once[2,3,4,5]. Having limited numbers of experimental units requires the study to be repeated multiple times, thus adding additional variables such as weather conditions, age or stage of production of animals, and perhaps bedding materials produced in different growing seasons.

With no known lab-scaled model to study factors affecting air quality and nutrient composition of the manure/bedding mixture resulting from beef deep-bedded mono-slope facilities, researchers first attempted to utilize commercial cattle facilities using a deep-bedded system[6,7,8]. Static flux chambers were used to measure NH_3 concentrations on the surface of mono-slope deep bedded cattle facilities over an 18 month period[6]. Two pens in each of two barns were measured. Chopped corn stalks were the preferred bedding material, but wheat straw and soybean stalks were also used for bedding during brief periods of this project. Bedding use ranged from 1.95 - 3.37 kg per animal per day and pen density ranged from 3.22 - 6.13 m^2 per animal. Subsequent studies measured ammonia and hydrogen sulfide emissions from the barn[7], and particulate matter concentrations outside the barn[8]. These studies were conducted over a 2 year period using two to four barn locations. The challenge with on-

farm data collection is the lack of control that the research has over the system. Producers change cattle diets, move animals from pen to pen, use bedding materials from different sources, and clean and re-bed pens as their production and labor force allows, thus confounding many variables. On-farm research also involves travel expenses and large quantities of experimental treatments (such as bedding material). The objective of this project was to develop a lab-scale model that could be used to study factors affecting air quality and nutrient management in cattle deep-bedded mono-slope facilities.

Protocol

The study is designed to be conducted over 42 days with weekly data collection. All animal procedures were reviewed and approved by the US Meat Animal Research Center Institutional Animal Care and Use Committee.

1. Constructing Simulated Bedded Packs

1. Begin with plastic cylinder containers that are 0.42 m high with a 0.38 m diameter.
   NOTE: In this study, one particular 10-gallon commercial trash container was used (see **Table of Materials**), but other similar-sized plastic containers would be suitable.
2. Drill six 1-cm holes equally spaced around the circumference of the plastic container into each plastic container approximately 5 cm the top of the plastic container. Remove any plastic remnants from the container.
3. Tare the plastic container and record the mass on the side of the plastic container. Weigh 320 g of selected bedding material into weigh pan using a balance and add bedding material to the plastic container.
   NOTE: Any bedding material deemed suitable for use in livestock facilities can be used[9,10,11,12,13,14,15]. For modeling deep bedded cattle facilities in the Upper Great Plains, corn stover is considered the most common bedding material[1] but soybean stover, wheat straw, and wood chips have also been used[1]. If using this system to model deep-bedded swine or dairy facilities, wheat straw, barley straw, oat straw, hay, wood shavings, wood chips, sawdust, newspaper, corn cobs, soybean stubble, rice hulls, or sand may be more suitable[16,17,18].
4. Weigh 320 g of fresh cattle feces on a plastic plate using balance and add to the plastic container.
   NOTE: Urine and feces are collected and maintained as previously described[11].
5. Measure 320 mL of fresh cattle urine in 1000-mL graduated cylinder. Empty contents into the plastic container. Using a stirring rod (5.08-cm circumference), mix the bedding material mixture slightly for 30 s.
   NOTE: In this case, a hollow steel rod with a plastic cover on the end was used. Alternatively, any type of rod could be used.
6. Clean the end of the stirring rod between each bedded pack using an antiseptic disposal wipe to prevent cross-contamination of microbes.
   NOTE: A bucket of warm soapy water can also be used to clean the stirring rod. A plastic sandwich bag can also be secured with a rubber band to the end of the rod and replaced after each bedded pack to prevent cross contamination.
7. Weigh and record the final mass of the bedding mixture. Place the plastic container in the environmental chamber[19] set to an ambient temperature of 18 - 20 °C with a dew point of 12 °C.

2. Maintaining the Simulated Bedded Packs

1. Forty-eight hours before adding feces and urine, remove frozen feces and urine from freezer and allow to thaw at room temperature (20 - 25 °C).
2. Less than an hour before adding urine to bedded pack, measure the pH of the urine.
3. Put on appropriate personal protective equipment (gloves, safety glasses) necessary for handling 6 M NaOH.
4. Pour 25 mL of 6 M sodium hydroxide (NaOH) into the graduated cylinder. Stir the mixture, then test the pH using a pH probe. Repeat until the urine reaches pH 7.4, physiological pH[20].
5. Once the pH of the urine is adjusted, replace cap on the urine container when not in use to prevent volatilization of nitrogen from urine.
6. Weigh and record the mass of the bedded pack. If fresh bedding is to be added on this day, weigh 320 g of selected bedding material into aluminum pan using balance and add bedding material to the respective bedded packs. If no bedding is to be added on this day, continue to Step 2.7.
7. Weigh 320 g of thawed cattle feces on a plastic plate using balance and add to the bedded pack.
   NOTE: On Day 21, use fresh feces instead of thawed feces.
8. Measure 320 mL of thawed cattle urine in 1000-mL graduated cylinder. Empty contents onto the bedded pack.
   NOTE: On Day 21, use fresh urine instead of thawed urine.
9. Using a stirring rod, stir the bedding pack mixture slightly for 30 seconds. Clean the plastic end of the stirring rod between each bedded pack to prevent cross-contamination of microbes. Weigh and record the final mass of the bedding mixture.
10. Return the plastic container in the environmental chamber.
11. Repeat steps 2.1 - 2.10 on Monday, Wednesday, and Friday of each week, with bedding material being added (Step 2.6) and air samples collected each Wednesday.

3. Collecting Samples from the Simulated Bedded Packs

NOTE: Samples are collected from the simulated bedded packs once weekly, prior to adding feces, urine, and fresh bedding.

1. **Preparing to collect air samples from headspace of each simulated bedded pack.**
   1. Turn on all air sampling equipment and allow to warm up according to manufacturer's directions, approximately 1 hour.
      NOTE: See **Table of Materials** for ammonia (NH_3), hydrogen sulfide (H_2S), methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) gas analyzers used in this study.
   2. Measure the distance from the top of the simulated bedded pack to the top of the plastic container holding the simulated bedded pack using a ruler.

3. Calculate the volume of the headspace area using the following formula:

$$V = \pi r^2 h + V_{flux\ chamber}$$

where r = radius of the plastic container,
h = distance from the top of the bedded pack to the top of the plastic container, and
$V_{flux\ chamber}$ = volume of the flux chamber located on top of the plastic container.
NOTE: The flux chambers used in this study had an internal volume of $0.007 m^3$ with a surface area of $0.064\ m^2$ [21,22].

4. Push a metal stake approximately 5 cm into surface of the bedded pack at the approximate center of the pack. Thread 0.64-cm inert tubing through one of the 1-cm holes at the top of each simulated bedded pack container and secure on a 12.5-cm metal stake 1.3 cm above the surface of the bedding pack. Place stainless steel hemispherical static flux chambers[21,22] with rubber skirts on top of each simulated bedded pack (**Figure 1**).
NOTE: Rubber skirts are 61-cm squares made of soft, elastic rubber with 22.9-cm diameter holes cut in the center. The hole fits over the flux chamber and the skirts form a seal on the top of the plastic container when placed on the container.

5. Attach 0.64-cm inert tubing to the flux chambers using inert compression fittings.
NOTE: The inert tubing is attached to the gas sampling manifold which feeds into the air sampling equipment. The gas sampling system is controlled by a 24-volt Programmable Logic Relay (see **Table of Materials**) which signals multi-positional 3-way solenoids to open and close one of eight air inlet lines on the gas sampling manifold. One line is opened at a time to allow for individual air sampling from each bedded pack.

6. Begin flushing ambient air from the room through the tubing at a rate of 5 L min^{-1} for 30 minutes.
NOTE: See **Table of Materials** for pump used to flush the air through the sample lines.

2. **Measure concentration of ammonia, carbon dioxide, methane, and hydrogen sulfide in headspace of simulated bedded packs.**
   1. After adequately flushing the simulated bedded packs, open stopcock on sample line to draw ambient air from the room into inert sample lines connected to gas sampling manifold.
   2. Activate the programmable logic relay to begin pulling air into the air sampling equipment. Record measurements from ambient air for 20 minutes to determine concentration of measured gases in ambient air. This will be used as a background air concentration. When finished collecting ambient air concentration, close the stopcock on the sample line.
   3. Activate the programmable logic relay to begin sampling air from the inert sample lines attached to each flux chamber. Record measurements from each sample line for 20 minutes to determine concentrations of measured gases in the headspace of each bedded pack.
   4. Results can be reported as the average concentration of the gas (NH_3, CO_2, N_2O, CH_4, H_2S) in the air samples (mg kg^{-1} or ppm), or the flux density (emission rate) of the gas can be calculated on a mass per unit area per unit time basis using the following equation:

$$J = \frac{Q C_{air}}{A}$$

where J = the flux in µg m^{-2} min^{-1},
A = the area of the source (m^2) inside the chamber,
Q = the sweep air flow rate m^3 min^{-1}, and
C_{air} = the VOC concentration leaving the chamber (µg m^{-3})[23].

3. **Measure concentration of odorous volatile organic compounds in the headspace of simulated bedded packs.**
   1. Put on latex or nitrile disposable gloves.
   2. After adequately flushing the simulated bedded packs, remove brass storage caps from preconditioned stainless steel sorbent tubes.
   NOTE: The sorbent tubes used in this study were 89 mm × 6.4 mm OD filled with Tenax TA sorbent (see **Table of Materials**). Brass caps have polythtrafluorethylene (PTFE) ferrules.
   3. Attach the scored end of the sorbent tube to the inlet port on the flux chamber using flexible rubber tubing, and the other end of the sorbent tube to a vacuum pump.
   NOTE: The vacuum pump used in this study (see **Table of Materials**) pulled air through the sorbent tubes at a flow rate of 75 mL min^{-1}.
   4. Allow the pump to pull air into the sorbent tube for 5 min for a sample volume of 0.375 L, then turn off pump and disconnect sorbent tube. Replace the brass storage caps on the ends of the sorbent tubes.
   5. Repeat Steps 3.3.1 - 3.3.4 to collect one sorbent tube for each bedded pack.
   6. Store sorbent tubes until analysis by thermal desorption-gas chromatograph-mass-spectrometry (TD-GC-MS). Tubes may be stored at room temperature (20 - 25 °C) for <24 h. If storing >24 h, store in refrigerator.
   7. Immediately before sample analysis on the TD-GC-MS system, remove brass storage caps from sorbent tubes and replace with PTFE analytical caps[23].
   8. Analyze sorbent tubes for volatile organic compounds[24] (acetic acid, butyric acid, propionic acid, isobutyric acid, isovaleric acid, valeric acid, hexanoic acid, heptanoic acid, phenol, p-cresol, indole, skatole, dimethyl disulfide, and dimethyl trisulfide) using TD-GC-MS[23,24,25].
   9. Results can be reported as concentration of the VOC in the air samples (µg m^{-3}), or the flux density (emission rate) of the VOC can be calculated on a mass per unit area per unit time basis using the following equation:

$$J = \frac{Q C_{air}}{A}$$

where J = the flux in µg m^{-2} min^{-1},
A = the area of the source (m^2) inside the chamber,
Q = the sweep air flow rate m^3 min^{-1}, and
C_{air} = the VOC concentration leaving the chamber (µg m^{-3})[23].

4. **Collect physical and chemical measurements of the simulated bedded packs.**
   NOTE: Temperature, pH, and evaporative water loss are measured each time additional materials were added to the simulated bedded packs. Nutrient composition is determined at Day 0 and Day 42. Free air space is determined at Day 42 only.

1. Determine the temperature of the bedded pack by inserting a temperature probe into the center of the bedded pack, approximately 7.6 cm below the surface of the simulated bedded pack. Allow the temperature to stabilize and record.
2. Determine estimated evaporative water loss
   1. Place the plastic container on the balance.
   2. Measure and record the mass of the simulated bedded pack before and after each addition of feces/urine/bedding to the simulated bedded pack.
   3. Calculate the estimated evaporative water loss by subtracting the current day's beginning mass from the previous day's ending mass. The difference is the estimated mass of water that evaporated from the bedded pack between the days and can be used to compare relative differences between bedded pack, although it does not reflect absolute loss.

3. Determine pH of simulated bedded pack
   1. Collect a representative 5 - 10 g sample from each simulated bedded pack from the center of the pack at a depth of approximately 7.6 cm below the surface of the bedded pack. Put the sample in a plastic 50-mL conical tube, cap, and label.
   2. Calibrate the pH meter with buffers pH 4 and 7 according to manufacturer's directions.
   3. Determine the mass of each conical.
   4. Dilute each sample 1:2 on a mass basis with distilled, deionized water. Shake the conical to mix the water and bedding material. Insert the pH probe into the conical, measure, and record the pH of the sample.

4. On Days 0 and 42 only, determine nutrient content of the simulated bedded pack.
   1. Collect a 50 g representative sample from each simulated bedded pack from the center of the pack at a depth of approximately 7.6 cm below the surface of the bedded pack. Place in a paper soil sample bag.
   2. Transport to a laboratory for nutrient analysis within 24 hours. Store in refrigerator until samples can be transported to a laboratory for nutrient analysis.
   NOTE: Any macro or micro nutrient can be analyzed. We analyze for total nitrogen[26], phosphorus and sulfur analysis[27] at a commercial laboratory.

5. On Day 42 only, determine free air space in simulated bedded pack.
   1. Place the plastic container on a balance and record the mass. Slowly fill with water until the surface of the water is even with the surface of the simulated bedded pack. Allow water to settle until no more bubbles are coming from the simulated bedded pack, then record the mass of the plastic container
   2. Determine the percentage of free air space using the following calculation:

$$\% \ Free \ Air \ Space = (Mass_{water \ added}/Mass_{total}) * 100\%$$

5. After completing all desired data collection steps (Steps 3.1 - 3.4), add feces, urine, and bedding to the simulated bedded packs following Steps 2.1 - 2.10.

Representative Results

To date, seven research studies have been published[9,10,11,12,13,14,15] using this procedure, with modifications and adjustments made to improve the model and reflect objectives of the specific experiments. This procedure has been used to evaluate the effect of numerous bedding materials and ambient temperature on odor and gas production, as well as amendments that can be added to control ammonia emissions. Chemical and physical properties of the bedded packs have been measured in commercial barns[6,28] as well as in the simulated bedded packs (**Table 1**). This data was used to determine if the protocol was a suitable model to supplement expensive on-farm research trials. Air quality data has been collected from commercial facilities and simulated bedded packs using two different methods (**Table 2**). The gas sampling system described in this protocol is new technology that has been tested and compared to previously used methods.

The dry matter composition of simulated bedded packs were within the range of published dry matter content of bedded pack material collected from commercial facilities[6,28]. The first time the protocol was used[11], 400 g of bedding were initially added to the bedded packs with subsequent additions of 200 g per week of fresh bedding, and 400 g each of urine and feces added three times weekly. This was set up to simulate commercial barns in which multiple bales of bedding are added initially and only one or two bales added to the pack per week thereafter. The ratio of bedding:livestock waste was estimated using data collected from commercial deep bedded mono-slope facilities[1,6]. At the end of the first study, the dry matter content of the bedded packs was similar to the dry matter content measured in bedded pack material collected from commercial facilities[6,28]. However, visual observation of the bedded packs indicated that there was a lot of variability in the water holding capacity of the bedding materials. For example, bedded packs with corn cobs appeared very wet, but had a dry matter content of 27.2 ± 1.5%[16], while bedded packs with wheat straw bedding appeared relatively dry, but had a dry matter content of 21.2 ± 1.1%[11]. To try to increase the dry matter content of the bedded packs to better represent commercial barns[6,28], the protocol was adjusted slightly with 320 g each of bedding, urine, and feces added when the pack was started, three weekly additions of 320 g each of urine and feces, and one weekly addition of 320 g of bedding material added to the packs. This protocol raised the dry matter content of the bedded packs, but was highly dependent upon the bedding material[13] used in the experiment and the temperature of the environmental chambers[14]. Even though it was variable, the dry matter content of the simulated bedded packs were within the range measured in commercial barns so the second protocol has been used for all subsequent studies.

The nutrient composition, pack temperature, and pH of the simulated bedded packs provide further evidence that the simulated bedded packs are a good model to represent manure bedded packs in commercial facilities. Total N, total P, total S, and total K have consistently been within the range of the nutrient content measured from commercial deep-bedded mono-slope facilities[6,28]. Partial composting occurs in the bedded packs of deep-bedded mono-slope facilities, so it was important to replicate the temperature of commercial facilities in the lab-scaled simulated bedded packs. Temperature of bedded packs in deep-bedded commercial facilities when ambient air temperature was between 0 and 20.6 ˚C was 19.2 ± 0.3 ˚C [6]. The temperature in the environmental chambers was set at 20 ˚C for most of the studies conducted using this protocol. In these studies, the temperature of the simulated bedded packs has consistently been between 18.3 and 20.1 ˚C. The exception to this was when temperature was a factor that was tested in a three-way factorial experiment. Two environmental chambers were set at 40 ˚C and two were set at 10 ˚C. In that study, the temperature of the simulated bedded packs was 12 - 13 ˚C in the cold chambers and 32 - 35 ˚C in the warmer chambers. Once again, this reflected commercial barns, where pack temperatures were 15.4 ± 0.4 ˚C when ambient temperatures were 0 ˚C or colder, and 29.0 ± 0.3 ˚C when ambient air temperature was greater than 20.6 ˚C [6]. The pH of the bedded packs in commercial barns using corn stover bedding was measured in one study[6] and ranged from 7.5 - 8.0. Simulated bedded packs with corn stover bedding have had pH values of 7.1 - 7.3[11,13]. The pH of all simulated bedded packs has ranged from 6.2 to 9.0, which reflects a variety of bedding materials used in the experiments.

The gas sampling system that is used in this protocol was adapted from a series of studies conducted in commercial poultry, swine, and dairy barns as part of the National Air Emissions Monitoring Study[29]. This system flushes room air through the flux chamber, creating a dynamic flux chamber that measures the concentration of the selected gases that are emitted over a 20-minute period. Previous to using the gas sampling system, the steady-stead concentration of NH_3 was determined by collecting air samples from each bedded pack using static flux chambers with acid traps containing 2 mol L^{-1} sulfuric acid[6,22]. The air within the chamber was recycled through the acid traps at a rate of 1 L min^{-1} for 20 minutes. Total reduced sulfides were collected using a hand-held sampler. Air samples were recirculated through the static flux chambers using a small pump at a flow rate of 1 L min for no longer than 4 minutes. A minimum of four consecutive samples were drawn from each simulated bedded pack. Greenhouse gas concentrations (N_2O, CO_2, and CH_4) were determined by collecting one 20 mL sample of air from each simulated bedded pack using the septa at the top of each static flux chamber. The samples were later analyzed using a gas chromatograph. The previous method of collecting all these gas samples was very labor intensive and required two or three people to manage all the collection equipment. Use of the gas sampling system is much less labor intensive. One person is able to set up the gas sampling system, initiate the programmable logic relay and return approximately 160 minutes later when the samples have finished collecting gas data from 8 simulated bedded packs.

Results from the previous, labor-intensive sampling protocol are shown, as well as results from the gas sampling system (**Table 2**). Because of the amount of labor required to collect the data, not all data was able to be collected from commercial facilities. Ammonia concentration was collected using the acid-trap method from the surface of bedded packs in commercial mono-slope facilities and compared to the simulated bedded packs. Ammonia concentrations measured in the simulated bedded packs were consistently similar to NH_3 concentrations measured from bedded packs in commercial cattle facilities. Ammonia concentrations using the new gas sampling system appear to be on the low end of the concentrations present in cattle facilities. That may be caused by the NH_3 analyzer or it may be a reflection of the treatments in the experiments that used the new gas sampling system. It could also reflect a higher rate of air flow over the simulated bedded packs compared to the air flow in the commercial barns, which would dilute the concentration of the ammonia samples. One series of experiments tested the use of alum as a surface amendment that can be applied to the bedded packs to lower the pack pH, thereby reducing volatilization of nitrogen as NH_3. Carbon dioxide, CH_4, and N_2O have not been measured at the surface of a bedded pack in commercial facilities. However, the range of concentrations of these gases measured in simulated bedded packs using the previous gas chromatography method and the range of concentrations measured using the gas sampling system are very similar. Somewhat higher concentrations were produced when the simulated bedded packs were housed in a 35 ˚C environmental chamber compared to 20 ˚C chamber, which accounts for the variability among experiments. Comparing TRS to hydrogen sulfide is not a direct comparison, since TRS includes more than just hydrogen sulfide. Therefore, it is not surprising that TRS concentrations from simulated bedded packs are slightly higher than H2S concentrations measured using the gas sampling system. This is also a reflection of the studies conducted using the two sampling protocols. Bedded packs that contained green cedar bedding generated very high TRS concentrations[12], while those that contain corn stover bedding did not. The samples collected using the gas sampling system have used corn stover, wheat straw, soybean stover, and pine chip bedding materials but no green cedar bedding.

	Commercial Barns[1-2]		Simulated Bedded Packs[3-6]			
Dry matter,%	29.99 ± 3.15	16.0 – 36.6	20.8 – 27.2	22.3 – 26.1	24.0 – 58.0	20.8 – 24.9
Total N,g kg^{-1}	60.97 ± 13.77	21.2 – 23.6	19.4 – 28.2	17.8 – 22.3	15.6 – 18.6	17.8 – 23.8
Total P, g kg^{-1}	14.13 ± 3.99	6.7 – 7.5	6.2 – 9.6	7.1 – 9.6	6.7 – 8.5	6.2 – 9.6
Total S, g kg^{-1}	7.88 ± 1.48	5.6 – 6.7	3.6 – 6.5	4.5 – 5.3	---	3.6 – 6.5
Total K, g kg^{-1}	32.74 ± 8.39	15.5 – 21.1	16.3 – 23.1	---	18.8 – 25.6	16.3 – 25.2
Lignin, g kg^{-1}	---	---	26.5 – 139.6	49.9 – 136.9	---	62.6 – 139.6
Ash, g kg^{-1}	---	154 - 214	119.3 – 200.5	98.9 – 223.6	---	119.3 – 200.5
C:N Ratio	---	---	17.4 – 28.2	20.2 – 29.7	---	20.6 – 27.5
pH	---	7.5 - 8	6.2 – 7.2	6.8 – 7.6	8.5 – 9.0	7.4 – 7.7
Temperature, ˚C	---	15.4 – 29.0	18.3 – 19.9	18.4 – 20.0	12.0 – 35.0	19.7 – 20.1

[1]Euken, 2009. Standard deviation as reported by Euken, 2009 is shown. Total P and total K were calculated by converting reported P_2O_5 and K_2O composition, respectively.

[2]Spiehs et al., 2011. Data collected from two pens in each of two barns . Chopped corn stalks were the preferred bedding material, but wheat straw and soybean stalks were also used for bedding during brief periods of this project. Bedding use ranged from 1.95 – 3.37 kg per animal per day and pen density ranged from 3.22 – 6.13 m2 per animal.

[3]Spiehs et al., 2012. Data collected from simulated bedded packs. Bedding materials included corn stover, bean stover, wheat straw, pelleted corn cobs, paper, wood chips, and sawdust.

[4]Spiehs et al., 2014b. Data collected from simulated bedded packs. Bedding material included corn stover, pine wood chips, wet cedar chips, and dry cedar chips.

[5]Ayadi et al., 2015b. Data collected from simulated bedded packs using corn stover and bean stover bedding material. Two temperatures were used (40˚C and 10˚C)

[6]Spiehs et al., 2017. Data collected from simulated bedded packs using mixtures of bedding material containing 0, 10, 20, 30, 40, 60, 80, 100% pine with the remaining being corn stover.

Table 1. Range of reported dry matter and nutrient composition (dry-matter basis) of bedding/manure material from commercial deep bedded mono-slope facilities (Euken, 2009 and Spiehs et al., 2011) and from studies conducting using the simulated bedded packs (Spiehs et al., 2012, 2014, 2017 and Ayadi et al., 2015).

	Static flux chamber method[1]				Dynamic flux chamber method[2]
Ammonia, ppm	95.8 – 641.1	350.8 – 516.7	381 - 1584	386.3 – 502.3	89.4 – 166.7
TRS, ppb	---	8.2 – 165.9	---	5.3 – 11.4	---
Hydrogen Sulfide, ppb	---	---	---	---	0.1 – 18.1
Carbon dioxide, ppm	---	1232 - 2000	2322 - 6917	918 - 1158	957 – 2149
Methane, ppm	---	2.3 – 3.6	7.2 – 87.0	4.4 – 6.7	3.2 – 16.7
Nitrous oxide, ppm	---	0.67 – 0.72	0.31 – 0.77	0.21 – 0.23	0.44 – 0.58

[1]Spiehs et al., 2011, 2014a, 2015a, 2016a. Data from these studies were collected using acid traps for ammonia, a hand-held sampler of total reduced sulfides, and one sample from the headspace of each simulated bedded pack analyzed on a greenhouse gas GC for carbon dioxide, methane, and nitrous oxide.

[2]This data represents three studies conducting using varying bedding materials and surface amendments to control odor and gas emission. These studies were conducting using the gas sampling system and are not yet published.

Table 2. Range of reported concentrations of ammonia, total reduced sulfides (TRS), hydrogen sulfide, carbon dioxide, methane, and nitrous oxide from commercial deep bedded mono-slope facilities (Spiehs et al., 2011) and from studies conducting using the simulated bedded packs (Spiehs et al., 2014a, 2016 and Ayadi et al., 2015).

Figure 1. Simulated bedded packs in plastic containers with stainless steel flux chambers and rubber skirts attached and ready for air sampling. The simulated bedded packs are located inside the environmental chambers. Please click here to view a larger version of this figure.

Discussion

The frequent addition of urine and feces to the bedded packs is a critical step. We experimented with adding urine and feces just once weekly, but found that the bedded pack developed a crust, which trapped gases inside the pack and was not representative of commercial facilities. The use of fresh feces at the beginning of the study ensures that the bedded packs is inoculated with common bacterial populations found in cattle facilities. It is also important, when adding the urine, to remember to adjust the pH to physiological pH prior to adding to the bedded packs. On one occasion, an error was made and low pH urine was added to the bedded packs. This killed the methanogenic bacteria population. When setting up the gas sampling system, all fittings need to be secure to prevent leakage that may compromise the quality of the gas measurements.

The protocol has been adapted since it was first developed. Adapting the static flux chamber to be dynamic flux chambers allows researchers to calculate emissions instead of just concentrations in headspace gases. The use of new dynamic gas sampling systems also allows the sampling to be completed by one person instead of needing two to three people to manage all the data collection.

Adaptations could be made to use the simulated bedded packs to evaluate bedding materials or odor amendments used in swine or dairy facilities. Adjustments would need to be made to determine appropriate bedding:manure ratios typical in swine or dairy facilities. Published literature should provide dry matter and nutrient composition expectations from commercial swine or dairy facilities that would help estimate the amount of bedding, feces, and urine that would need to be needed to adjust the simulated bedded pack protocol to represent a swine or dairy facility. The protocol has never been used to measure an inorganic bedding material, such as sand, which is often used in dairy facilities. While there is no reason to believe it would not successfully measure gas emissions from a bedded pack containing inorganic bedding material, this would require additional testing.

There may be additional gases that could be sampled that we have not evaluated. Any gas sampling instrument that can be attached to an inert gas sampling line should, theoretically, be able to be used with this system.

The model could also be adjusted to explore different bedding:manure ratios if a researcher chose to do so. Perhaps a researcher was interested in determining the maximum amount of manure or urine that could be added to a bedded pack before significant odors were detected. Or a researcher wanted to examine different temperature and humidity effects on air quality. The model could also be adjusted to examine these factors.

The protocol was developed to measure air quality and nutrient composition from lab-scaled bedded packs in a controlled environment and has been used to effectively evaluate many different bedding materials, environmental variables (temperature, humidity), and potential mitigation treatments that can improve air quality in commercial deep-bedded mono-slope facilities. The model is dynamic and allows researchers to easily collect many chemical and physical measurements from the bedded pack, including NH_3, CH_4, N_2O, CO_2, H_2S, VOC, temperature, pH, nutrient composition, free air space, and potentially others that have not yet been measured. Weekly measurements collected over the course of six to seven weeks allows sufficient time to see changes in air quality measurements over time as the bedded pack mature. The data collected from the simulated bedded packs is within the range of concentrations previously measured in commercial deep-bedded mono-slope facilities. Past studies have demonstrated that that 8 - 10 experimental units per treatment are sufficient to detect statistical differences among the simulated bedded packs[9,10,11,12,13,14,15]. The bedded packs are easy to maintain, requiring less than 10 minutes of labor per bedded pack per week to add urine, feces, and bedding. Sample collection using the gas sampling system requires 20 - 30 minutes per bedded pack, depending on the measurements that are being collected. In the past, as many as 20 bedded packs have been analyzed by one person in a normal 8-hour workday. The use of lab-scaled bedded packs allows the researcher to control variables such as temperature, humidity, and bedding source

that are difficult or impossible to control in a research or commercial facility. Several lab-scale studies can be conducted to eliminate possible treatments before trying them in a research or commercial-sized facility.

The primary limitation of the model is that it is not a perfect simulation of "real-world" conditions. It is difficult to perfectly simulate commercial conditions such as continual additional of urine and feces which occurs in a livestock facility. Based on the dry matter content and nutrient composition of the simulated bedded packs compared to commercial facilities, and the labor available in our laboratory, we have determined thrice weekly additions of urine and feces to be sufficient. However, if a modification could be developed to periodically add fresh urine and feces multiple times daily, that would better simulate the commercial environment.

Another recognized limitation is the use of frozen and thawed feces and urine. While every effort is made to quickly freeze the urine and feces to prevent volatilization of the nitrogen and any bacterial growth, urine and feces collected from a balance study are only collected once daily. It takes an hour or longer to collect, weigh, reset the collection containers, and partition the urine and feces. It also requires several hours for a 20-L carboy of urine to completely freeze, even after being placed in a -4 °C freezer. During this time, volatilization and bacterial growth may occur. To compensate for this time delay between collection and freezing, the urine is acidified to pH 4 immediately after removing the container from the collection apparatus to prevent bacterial growth and nitrogen volatilization. The urine is restored to pH 7 once it is thawed, but this may not be exactly the same as adding fresh urine. However, as there have been no observed increases in NH_3 volatilization following the addition of fresh urine to the bedded packs compared to frozen urine, we believe we have minimized this limitation. Bacterial populations are killed or decreased when feces is frozen. This is a recognized limitation of the protocol that we have attempted to minimize by adding fresh feces on Day 0 and Day 21.

The use of a steel rod to gentle mix the freshly added feces and urine with the bedding material may not perfectly simulate the weight of the cattle in a commercial facility, thus resulting in somewhat different compaction and water holding capacity. To account for the porosity of the bedded backs, and as an indication of the free air space that may be present in the bedded pack, water was poured into the bedded packs at the end of each study to determine a percentage of free air space present in each bedded pack[9,10,11,12,13,14,15]. Free air space stayed generally uniform from one study to another, but has not been compared to the free air space that is present in a commercial facility.

The protocol has not been tested with other livestock species or facility types, such as swine deep-bedded hoops or Swedish deep-bedded farrowing facilities, dairy compost barns or other dairy bedded facilities, or any type of poultry facility using bedding. While it seems that the model would have potential to be used as a model for other livestock facilities, adjustments to the protocol may be necessary to adequately represent any facility beyond a beef cattle deep-bedded facility.

While the model is not a perfect simulation of commercial facilities, it can offer a starting point when evaluating factors such as bedding, temperature, humidity, or amendments that can be added to a bedded pack in a livestock facility. It allows a researcher to evaluate treatment differences in a controlled environment and eliminate potentially less effective treatment options before spending money on the resources necessary for a full-scale commercial-size operation.

Disclosures

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References

1. Doran, B., Euken, R., Spiehs, M. Hoops and mono-slopes: What we have learned about management and performance. In: *Feedlot Forum 2010. Ames, Iowa: Iowa State University, Iowa Beef Center.* p. 8-16 (2010).
2. Andersson, M. Performance of bedding materials in affecting ammonia emissions from pig manure. *J. Agric. Engng. Res.* **65**, 213-222 (1996).
3. Jeppsson, K.H. Volatilization of ammonia in deep-litter systems with different bedding materials for young cattle. *J. Agric. Engng. Res.* **73**, 49-57 (1999).
4. Powell, J.M., Misselbrook, T.H., Casler, M.D. Season and bedding impacts on ammonia emissions from tie-stall dairy barns. *J. Environ. Qual.* **37**, 7-15 (2008).
5. Gilhespy, S.L., Webb, J., Chadwick, D.R., Misselbrook, T.H., Kay, R., Camp, V. Retter, A.L, Bason, A. Will additional straw bedding in buildings housing cattle and pigs reduce ammonia emissions? *Biosystems Engng.* **102**, 108-189 (2009).
6. Spiehs, M.J., Woodbury, B.L., Doran, B.E., Eigenberg, R.A., Kohl, K.D., Varel, V.H., Berry, E.D., Wells, J.E. Environmental conditions in beef deep-bedded mono-slope facilities: A descriptive study. *Trans ASABE.* **54**, 663-673 (2011).
7. Cortus, E.L., Spiehs, M.J., Doran, B.E., Al Mamun, M.R.H., Ayadi, F.Y., Cortus, S.D., Kohl, K.D., Pohl, S., Stowell, R., Nicolai, R. *Ammonia and hydrogen sulfide concentration and emission patterns for mono-slope beef cattle facilities in the Northern Great Plains.* Paper No. 141897896. St. Joseph, MI: ASABE (2014).

8. Spiehs, M.J., Cortus, E.L., Holt, G.A., Kohl, K.D., Doran, B.E., Ayadi, F.Y., Cortus, S.D., Al Mamun, M.R., Pohl, S., Nicolai, R., Stowell, R., Parker, D. Particulate matter concentration for mono-slope beef cattle facilities in the Northern Great Plains. *Trans. ASABE.* **57**, 1831-1837. (2014).

9. Ayadi, F.Y., Cortus, E.L., Spiehs, M.J., Miller, D.N., Djira, G.D. Ammonia and greenhouse gas concentrations at surfaces of simulated beef cattle bedded manure packs. *Trans. ASABE.* **58**, 783-795. (2015).

10. Ayadi, F.Y., Spiehs, M.J., Cortus, E.L., Miller, D.N., Djira, G.D. Physical, chemical, and biological properties of simulated beef cattle bedded manure packs. *Trans. ASABE.* **58**, 797-811 (2015).

11. Spiehs, M.J., Brown-Brandl, T.M., Parker, D.B., Miller, D.N., Berry, E.D., Wells, J.E. Effect of bedding materials on concentration of odorous compounds and *Escherichia coli* in beef cattle bedded manure packs. *J. Environ. Qual.* **42**, 65-75 (2013).

12. Spiehs, M.J., Brown-Brandl, T.M., Parker, D.B., Miller, D.N., Jaderborg, J. P., Diconstanzo, A., Berry, E.D., Wells, J.E. Use of wood-based materials in beef bedded manure packs: 1. Effect on ammonia, total reduced sulfide, and greenhouse gas concentrations. *J. Environ. Qual.* **43**, 1187-1194 (2014).

13. Spiehs, M.J., Brown-Brandl, T.M., Berry, E.D., Wells, J.E., Parker, D.B., Miller, D.N., Jaderborg, J. P., Diconstanzo, A. Use of wood-based materials in beef bedded manure packs: 2. Effect on odorous volatile organic compounds, odor activity value, *Escherichia coli*, and nutrient concentration. *J. Environ. Qual.* **43**, 1195-1206 (2014).

14. Spiehs, M.J., Brown-Brandl, T.M., Parker, D.B., Miller, D.N., Berry, E.D., Wells, J.E. Ammonia, total reduced sulfides, and greenhouse gases of pine chip and corn stover bedding packs. *J. Environ. Qual.* **45**, 630-637 (2016).

15. Spiehs, M.J., Berry, E.D., Wells, J.E., Parker, D.B., Brown-Brandl, T.M. Odorous volatile organic compounds, Escherichia coli, and nutrient concentrations when kiln-dried pine chips and corn stover bedding are used in beef bedded manure packs. *J. Environ. Qual.* **46**, 722-732 (2017).

16. Herbert, S., Hashemi, M., Chickering-Sears, C., Weis, S. *Bedding options for livestock and equine.* University of Massachusetts Extension CDLE Pub. 08-5. (2008).

17. Honeyman, M.S., Patience, J.F. *Effects of bedding on pig performance.* Iowa State Research Farm Progress Reports. 143. http://lib.dr.iastate.edu/farms_reports/134 (2012).

18. Haverson, M., Honeyman, M.S., Adams, M.K. Swine system options for Iowa Swedish deep-bedded group nursing systems for feeder pigs production. *Extension and Outreach Publications 63.* http://lib.dr.iastate.edu/extension_pubs/63 (2006).

19. Brown-Brandl, T.M., Nienaber, J.A., Eigenberg, R.A. Temperature and humidity control in indirect calorimeter chambers. *Trans. ASABE.* **54**, 685-692 (2011).

20. Abney, C.S., Vasconcelos, J.T., McMeniman, J.P., Keyser, S.A., Wilson, K.R., Vogel, G.J., Galyean, M.L. Effects of ractophamine hydrochlodride on performance, rate and variation in feed intake, and acid-base balance in feedlot cattle. *J. Anim. Sci.* **85**, 3090-3098 (2007).

21. Miller, D.N., Woodbury, B.L. A solid-phase microextraction chamber method for analysis of manure volatiles. *J. Environ. Qual.* **35**,2383-2394 (2006).

22. Woodbury, B. L., Miller, D.N., Eigenberg, R.A., Nienaber, J.A. An inexpensive laboratory and field chamber for manure volatile gas flux analysis. *Trans. ASABE.* **49**, 767-772 (2006).

23. Koziel, J.A., Spinhirne, J.P., Lloyd, J.D., Parker, D.B., Wright, D.W., Kuhrt, F.W. Evaluation of sample recovery of malodorous livestock gases from air sampling bags, solid-phase microextraction fibers, Tenax TA sorbent tubes, and sampling canisters. *J. Air Waste Manag. Assn.* **55**, 1147-1157 (2005).

24. Parker, D.B., Gilley, J., Woodbury, B., Kim, K., Galvin, G., Bartelt-Hunt, S.L., Li, X., Snow, D.D. Odorous VOC emission following land application of swine manure slurry. *Atmos. Environ.* **66**, 91-100 (2013).

25. Parker, D. B., Koziel, J.A., Cai, L., Jacobson, L.D., Akdeniz, N. Odor and odorous chemical emissions from animal buildings: Part 6. Odor activity value. *Trans. ASABE.* **55**, 2357-2368 (2012).

26. Watson, M., Wolf, A., Wolf, N. *Total nitrogen In: John Peters, editor, Recommended methods of manure analysis.* Pub. A3769. Univ. of Wisconsin Cooperative Extension, Madison. p. 18-24. (2003).

27. Wolf, A., Watson, M. Wolf, N. Digestion and dissolution methods for P, K, Ca, Mg, and trace elements. In: John Peters, editor, *Recommended methods of manure analysis.* Pub. A3769. Univ. of Wisconsin Cooperative Extension, Madison. p. 30-38. (2003).

28. Euken, R. *A survey of manure characteristics from bedded confinement buildings for feedlot beef productions: Progress report.* Animal Industry Report Iowa State University, Ames, IA (2009).

29. Wang - Li, L., Li, Q.-F., Wang, K., Bogan, B.W., Ni, J.-Q., Cortus, E.L., Heber, A.J. The National Air Emission Monitoring Study's southeast layer site: Part I. Site characteristics and monitoring methodology. *Trans. ASABE.* **56**, 1157-1171 (2013).